

AMENDMENTS TO THE CLAIMS:

The following Listing of Claims replaces all prior Listings and versions of claims in the above-identified application.

Listing of Claims

1. (Currently Amended) A method to produce N-acetylglucosamine-6-phosphate glucosamine or N-acetylglucosamine by fermentation, comprising:
 - a) culturing in a fermentation medium a microorganism that is transformed with at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a glucosamine-6-phosphate acetyltransferase that has an amino acid sequence that is at least 95% identical to SEQ ID NO:30 ~~bacterium or yeast which comprises at least one genetic modification that results in the increased expression of a bacterial or yeast glucosamine-6-phosphate acetyltransferase; and~~
 - b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate[[,]] and N-acetylglucosamine.
- 2-3. (Canceled).
4. (Currently Amended) The method of Claim 1 [[3]], wherein the recombinant nucleic acid molecule further comprises a non-native promoter.
- 5-7. (Canceled).
8. (Currently Amended) The method of Claim 1 [[3]], wherein the glucosamine-6-phosphate acetyltransferase has the amino acid sequence ~~selected from the group consisting of~~ SEQ ID NO:30, ~~SEQ ID NO:32 and SEQ ID NO:34.~~

9. (Currently Amended) The method of Claim 1 ~~[[3]]~~, wherein expression of the recombinant nucleic acid molecule is inducible.

10. (Previously Presented) The method of Claim 9, wherein expression of the recombinant nucleic acid molecule is inducible by lactose.

11. (Canceled).

12. (Currently Amended) The method of Claim 10 ~~[[11]]~~, wherein the microorganism ~~genetic modification~~ further comprises a partial or complete deletion or inactivation of a gene encoding a LacI repressor protein.

13-20. (Canceled).

21. (Currently Amended) The method of Claim 1, wherein the microorganism ~~bacterium or yeast~~ further comprises ~~at least one a~~ a partial or complete deletion of an endogenous gene encoding a glucosamine-6-phosphate deaminase ~~in the bacterium or yeast that decreases the activity of glucosamine-6-phosphate deaminase in the bacterium or yeast.~~

22-24. (Canceled).

25. (Previously Presented) The method of Claim 1, wherein the step of culturing includes the step of maintaining the carbon source at a concentration of from about 0.5% to about 5% in the fermentation medium.

26. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising yeast extract.

27. (Previously Presented) The method of Claim 1, wherein the step of

culturing is performed in a fermentation medium comprising a carbon source selected from the group consisting of glucose, fructose, a pentose sugar, lactose and gluconic acid.

28. (Previously Presented) The method of Claim 27, wherein the pentose sugar is selected from the group consisting of ribose, xylose, and arabinose.

29. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising glucose and ribose.

30. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising glucose and gluconic acid.

31. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed at a temperature of from about 25°C to about 45°C.

32. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed at about 37°C.

33. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 4 to about pH 7.5.

34. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 6.7 to about pH 7.5.

35. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 4.5 to about pH 5.

36. (Currently Amended) The method of Claim 1, wherein the microorganism is a bacterium or yeast ~~is a bacterium~~.

37. (Currently Amended) The method of Claim 1, wherein the microorganism ~~is a bacterium or yeast is a yeast.~~

38. (Currently Amended) The method of Claim 37 [[36]], wherein the bacterium is a bacterium from a genus selected from the group consisting of: *Escherichia*, *Bacillus*, *Lactobacillus*, *Pseudomonas* and *Streptomyces*.

39. (Currently Amended) The method of Claim 37 [[36]], wherein the bacterium is a bacterium from a species selected from the group consisting of: *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Lactobacillus brevis*, *Pseudomonas aeruginosa* and *Streptomyces lividans*.

40. (Currently Amended) The method of Claim 251 [[37]], wherein the yeast is a yeast from a genus selected from the group consisting of: *Saccharomyces*, *Candida*, *Hansenula*, *Pichia*, *Kluveromyces*, and *Phaffia*.

41. (Currently Amended) The method of Claim 251 [[37]], wherein the yeast is a yeast from a species selected from the group consisting of: *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans*, *Hansenula polymorpha*, *Pichia pastoris*, *P. canadensis*, *Kluyveromyces marxianus* and *Phaffia rhodozyma*.

42-44. (Canceled).

45. (Currently Amended) The method of Claim 1, wherein the microorganism ~~bacterium or yeast~~ is transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a bacterial or yeast phosphoglucisomerase.

46. (Currently Amended) The method of Claim 45, wherein the phosphoglucisomerase comprises the amino acid sequence of SEQ ID NO:105.

47. (Currently Amended) The method of Claim 1, wherein the microorganism ~~bacterium or yeast~~ further comprises a partial or complete deletion of an endogenous gene encoding a phosphofructokinase in the bacterium or yeast.

48. (Canceled).

49. (Currently Amended) The method of Claim 1, wherein the microorganism ~~bacterium or yeast~~ has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a bacterial or yeast glutamine synthetase.

50. (Previously Presented) The method of Claim 49, wherein the glutamine synthetase comprises the amino acid sequence of SEQ ID NO:89.

51. (Canceled).

52. (Currently Amended) The method of Claim 1, wherein the microorganism ~~bacterium or yeast~~ has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a bacterial or yeast glucose-6-phosphate dehydrogenase.

53. (Previously Presented) The method of Claim 52, wherein the glucose-6-phosphate dehydrogenase comprises the amino acid sequence of SEQ ID NO:95.

54. (Currently Amended) The method of Claim 1, wherein the microorganism ~~bacterium or yeast~~ further comprises a partial or complete deletion of at least one endogenous gene encoding an enzyme involved in glycogen synthesis selected from the group consisting of: ADP-glucose pyrophosphorylase, glycogen synthase and a branching enzyme ~~in the bacterium or yeast~~.

55-56. (Canceled).

57. (Currently Amended) The method of Claim 1, further comprising wherein ~~the step of collecting comprises~~ recovering an intracellular product from the microorganism ~~bacterium or yeast~~ selected from the group consisting of: intracellular glucosamine-6-phosphate, glucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine and glucosamine, ~~or recovering an extracellular product from the fermentation medium selected from the group consisting of: glucosamine and N-acetylglucosamine.~~

58. (Currently Amended) The method of Claim 1, further comprising a step selected from the group consisting of:

- a) ~~purifying a product selected from the group consisting of glucosamine and N-acetylglucosamine from the fermentation medium;~~
- b) ~~dephosphorylating a product selected from the group consisting of glucosamine-6-phosphate and glucosamine-1-phosphate to produce glucosamine;~~
- e) ~~b) dephosphorylating a product selected from the group consisting of N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate to produce N-acetylglucosamine; and~~
- d) ~~c) treating a product selected from the group consisting of N-acetylglucosamine, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate to produce a glucosamine product selected from the group consisting of: glucosamine[.], and glucosamine HCl, glucosamine-6-phosphate and glucosamine-1-phosphate.~~

59. (Currently Amended) The method of Claim 58, wherein step (c) ~~(d)~~ comprises hydrolyzing the product selected from the group consisting of N-acetylglucosamine[.], and N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate, under acid and heat conditions or by enzymatic deacetylation.

60. (Previously Presented) The method of Claim 1, wherein N-acetylglucosamine produced by the fermentation method is recovered by precipitating N-acetylglucosamine-containing solids from the fermentation broth.

61. (Previously Presented) The method of Claim 1, wherein N-acetylglucosamine produced by the fermentation method is recovered by crystallizing N-acetylglucosamine-containing solids from the fermentation broth.

62-218. (Canceled).

219. (Currently Amended) A method to produce N-acetylglucosamine-6-phosphate glucosamine or N-acetylglucosamine by fermentation, comprising:

a) culturing in a fermentation medium a microorganism ~~bacterium or yeast~~ that expresses:

i) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate acetyltransferase that has an amino acid sequence that is at least 95% identical to SEQ ID NO:30 ~~and has glucosamine-6-phosphate acetyltransferase enzymatic activity~~; and

ii) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase that has an amino acid sequence that is at least 95% identical to the amino acid sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, ~~and~~ SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20; ~~and has glucosamine-6-phosphate synthase enzymatic activity~~; and

b) collecting a product produced from the step of culturing which is selected from the group consisting of ~~glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate~~[[,]] and N-acetylglucosamine.

220. (Previously Presented) The method of Claim 219, wherein the glucosamine-6-phosphate acetyltransferase has the amino acid sequence of SEQ ID NO:30.

221. (Previously Presented) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has the amino acid sequence of SEQ ID NO:6.

222. (Currently Amended) A method to produce N-acetylglucosamine-6-phosphate ~~glucosamine~~ or N-acetylglucosamine by fermentation, comprising:

- a) culturing in a fermentation medium an *E. coli* that expresses:
 - i) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate acetyltransferase that is at least 95% identical to ~~has~~ the amino acid sequence of SEQ ID NO:30; and
 - ii) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase that is at least 95% identical to ~~has~~ the amino acid sequence of SEQ ID NO:6; and
- b) collecting a product produced from the step of culturing which is selected from the group consisting of ~~glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate~~[[,]] and N-acetylglucosamine.

223. (Previously Presented) The method of Claim 229, wherein the *E. coli* further comprises a partial or complete deletion of *nagA*, *nagB*, and *nagE*.

224. (Previously Presented) The method of Claim 229, wherein the *E. coli* further comprises a partial or complete deletion of *manXYZ*.

225. (Previously Presented) The method of Claim 229, wherein the recombinant nucleic acid molecules of (a)(i) and (a)(ii) are inducible by lactose or

galactose.

226. (Previously Presented) The method of Claim 229, wherein the step of culturing is performed in a fermentation medium comprising glucose and fructose.

227. (Canceled).

228. (Currently Amended) The method of claim 219, wherein the microorganism ~~bacterium or yeast~~ further comprises a partial or complete deletion of an endogenous gene encoding a phosphofructokinase.

229. (Previously Presented) The method of claim 222, wherein the *E. coli* further comprises a partial or complete deletion of *pfkA*.

230. (Currently Amended) The method of Claim 228, wherein the microorganism ~~bacterium or yeast~~ further comprises a partial or complete deletion of endogenous genes encoding N-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, and N-acetyl-glucosamine-specific enzyme II^{Nag}.

231. (Currently Amended) The method of Claim 228, wherein the microorganism ~~bacterium or yeast~~ further comprises a partial or complete deletion of an endogenous gene encoding mannose transporter EIIM,P/III^{Man}.

232. (Previously Presented) The method of Claim 228, wherein the recombinant nucleic acid molecules of (a)(i) and (a)(ii) are inducible by lactose or galactose.

233. (Previously Presented) The method of Claim 228, wherein the step of culturing is performed in a fermentation medium comprising glucose and fructose.

234. (Previously Presented) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:4.

235. (Previously Presented) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:6.

236. (Previously Presented) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:8.

237. (Previously Presented) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:10.

238. (Previously Presented) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:12.

239. (Previously Presented) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:14.

240-242 (Canceled).

243. (New) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:16.

244. (New) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:18.

245. (New) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:20.

246. (New) The method of Claim 219, wherein the microorganism is a bacterium or yeast.

247. (New) The method of Claim 219, wherein the microorganism is a bacterium.

248. (New) The method of Claim 219, wherein the microorganism is a yeast.

249. (New) The method of Claim 222, wherein the glucosamine-6-phosphate acetyltransferase has the amino acid sequence of SEQ ID NO:30.

250. (New) The method of Claim 222, wherein the glucosamine-6-phosphate synthase has the amino acid sequence of SEQ ID NO:6.

251. (New) The method of Claim 1, wherein the microorganism is a yeast.

252. (New) The method of claim 1, further comprising the step of contacting the fermentation medium with at least one ion exchange resin.